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(71) Applicant *(for all designated States except US)*: ARIAD PHARMACEUTICALS, INC. [US/US]; 26 Landsdowne Street, Cambridge, MA (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: WEIGELE, Manfred [GR/US]; 6 Canal Park #601, Cambridge, MA 02141 (US). LOEWE, Mallory, F. [US/US]; 98 Choate Street, Essex, MA 01929 (US). POSS, Christopher, S. [US/US]; Apartment 37, 8 Tozer Road, Beverly, MA 01915 (US). LAZAROVA, Tsvetelina [BG/US]; Apartment 36, 10941 Strathmore Drive, Westwood, Los Angeles, CA 90024 (US).

(74) Agent: BERSTEIN, David, L.; Ariad Pharmaceuticals, Inc., 26 Landsdowne Street, Cambridge, MA 02139 (US).

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(57) Abstract

Novel compounds related structurally to Brefeldin A useful as antiviral, antifungal, antiproliferative, immunosuppressive and detoxifying agents as well as pharmaceutical compositions and methods based thereon are disclosed.

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New Protein Trafficking Inhibitors

BACKGROUND

Brefeldin A (decumbin, "BFA") was first isolated in 1958 as a fungal metabolite from Penicillium decumbens (Singleton, V. L., et al., Nature 181:1072-1073 (1958)). BFA has a molecular weight of 280.37 ($C_{16}H_{24}O_4$) and reportedly has a wide range of biological activities, including antifungal, antiviral and antitumor effects. See Betina, Folia Microbiol. 37(1):3-11 (1992) for a recent review. At the cellular level, BFA has dramatic effects on the secretory pathway and protein trafficking in mammalian cells. (Pelham, H. R. B., Cell, 67:449-451 (1991); (Klausner, R. D., et al., J. Cell Biol., 116:1071-1080 (1992)). BFA has been shown to also inhibit protein transport in fungi, such as Candida albicans (Arioka, M., et al., J. Gen. Microbiol., 137:1253-1262 (1991)) and inhibit the presentation of endogenous and exogenous protein antigens by MHC class II-restricted T-cells (Adorini, L., et al., Nature, 246:63-66 (July 1990)). BFA has also been shown to have selective cytotoxic activity against human tumor cell lines (Ishii, S., et al., J. Antibiot., XLII:1877-1878 (1989)).

BFA also inhibits virus replication by interfering with the intracellular transport and maturation of viral proteins. Inhibition, as defined herein, means a significant reduction in virus particle replication, as well as complete abrogation of virus particle replication. Enveloped viruses, such as herpes viruses (including Herpes Simplex) and Human Immunodeficiency Virus (HIV), require the host cell secretory apparatus for transport and processing of envelope (membrane) glycoproteins during the course of virus assembly and maturation. BFA has also been shown to inhibit infectious viral particle formation by preventing the transport of envelope glycoprotein to the cell surface as required for assembly of mature, infectious viral particles. (Cheung, P., et al., J. Virol., 65:1893-1904 (1991); Pal, R., et al., Aids Res. Human Retroviruses, 7:707-712 (1991); see also Takatsuki et al, Agric. Biol. Chem. 49(3):899-902 (1985)).

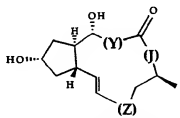
BFA has a short biological half-life. It is rapidly deactivated *in vivo* via conjugation with glutathione by glutathione S-transferase and subsequently transported out of the cell (Bruning, A., et al., J. Biol. Chem., 267:7726-7732

(1992)). Compounds having some or all of the biological activities of BFA combined with an extended useful biological half-life and/or improved overall therapeutic profiles would be valuable for the treatment of viral, bacterial, fungal and other diseases, as anti-cancer agents, as immunosuppresive agents and as detoxifying agents.

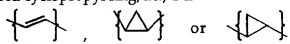
DESCRIPTION OF THE INVENTION

This invention concerns novel compounds related structurally to Brefeldin A; methods of synthesizing these compounds; use thereof as antiviral, antifungal, detoxification and antiproliferative agents (e.g., antitumor agents and agents to treat genital warts); pharmaceutical compositions which contain these compounds as active components; and pharmaceutical methods involving administration of these compounds to mammals, preferably human patients, in need thereof. These compounds block, or inhibit, the transport of proteins from the endoplasmic reticulum (ER) and through the Golgi apparatus in a cell and are also useful as experimental research reagents.

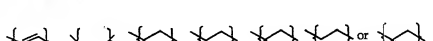
This invention encompasses compounds of the formula:



wherein J is O or a substituted or unsubstituted N, Y is a *trans* unsaturated 2-carbon unit or a fused cyclopropyl ring, i.e., Y is

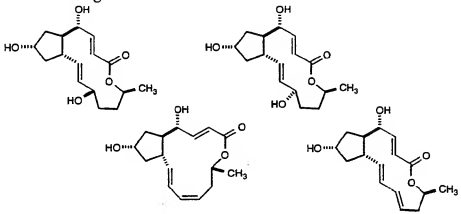


and Z is an unsaturated 2-carbon unit (*cis* or *trans*), or a hydroxy- or fluoro-substituted saturated 2-carbon unit, i.e., Z is

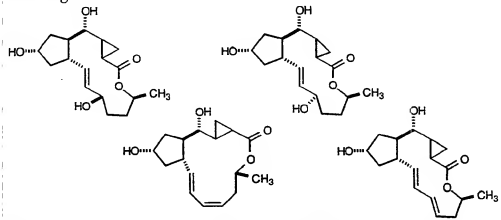


as well as their isolated diastereoisomers, diastereomeric mixtures and esters and polyethers thereof.

Illustrative compounds in which J is O and Y is a *trans* double bond include the following:



Illustrative compounds in which J is O and Y is a cyclopropyl ring include the following:



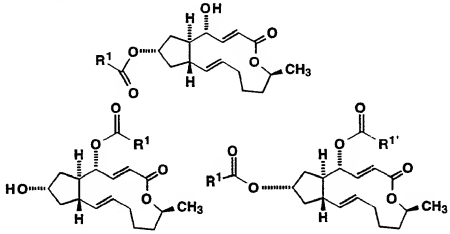
The cyclopropyl ring junctions may have either R,R or S,S stereochemical configuration.

The esters referred to above include among others compounds of this invention in which one or more hydroxyl goups bear acyl moieties of the formula R^1CO- where each R^1 is independently selected and may be alkyl, aryl or heteroaryl. In embodiments where R^1 is alkyl, R^1 may be lower alkyl (i.e., upto 6 carbons), optionally substituted with one or more halogen atoms, preferably fluorine. Where R^1 is aryl, it preferably contains a hydroxy, alkoxy or amino substituent, preferably in the para or ortho position. By way of illustration, compounds of this invention include, among others, compounds in which R^1 is

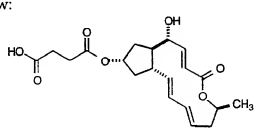
CH_3-, CF_3-, CH_2F-, CHF_2-, $CH_3(CH_2)_nCF_2$-, 3-pyridyl and Z-(C_6H_4)-, where Z is para- hydroxy, methoxy, amino, alkylamino or dialkylamino.

In other embodiments, $\mathbf{R^1}$ is -$(CH_2)_n$-L, where n is an integer from 2 through about 6 and L is -CO_2H, SO_3H, PO_3H, amino, alkylamino, dialkylamino or trialkylammonium and pharmaceutically acceptable salts thereof. The alkyl groups may be the same or different and may be substituted or unsubstituted and may be straight-chain, branched or cyclic. For example, alkyl substituents include saturated straight-chain, cyclic or branched hydrocarbon moieties, preferably of one to about twelve carbon atoms, including methyl, ethyl, n-propyl, i-propyl, cyclopropyl, n-butyl, i-butyl, t-butyl, cyclobutyl, cyclopropylmethylene, pentyl, hexyl, heptyl, octyl and so forth, and may be optionally substituted with one or more substituents such as lower alkoxy, carboxy, amino, phenyl, aryl, mercapto, halo (fluoro, chloro, bromo or iodo), azido and cyano.

Illustrative esters include the following (in which J is O and Y and Z are both trans C=C units):

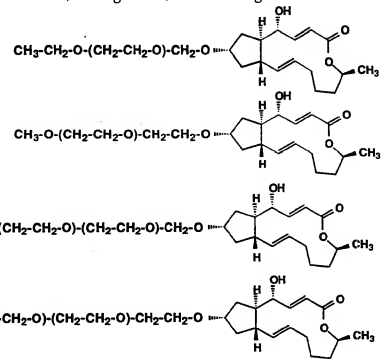


An illustrative example of such an ester, where $\mathbf{R^1}$ is (COOH)-CH_2CH_2-CO-, is depicted below:



Also encompassed are compounds of this invention in which one or more hydroxyl groups are substituted with a polyether moiety of the formula:

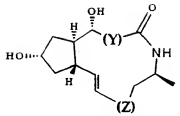
where R^2 is alkyl, preferably lower alkyl; n is 0, 1 or 2; and m is 1 or 2. Preferably R^2 is methyl or ethyl and n is 2. However, where R^2 is methyl, J is O and Z is a trans C=C unit, either n is 0 or 2, or m is 2. By way of illustration, compounds of this invention include, among others, the following:



Other compounds of this invention include lactams, acetals and ethers corresponding to the lactones described above.

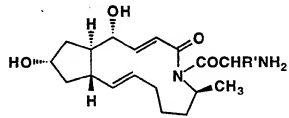
Such lactams have the general formula described above in which J is -NW- where W is -H, alkyl, aryl, arylalkyl, acyl or alpha aminoacyl. Further examples of suitable W moieties are disclosed below.

In the simplest members of this class, W is H:



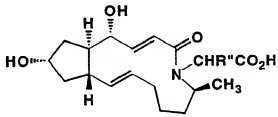
In other lactams of interest, W is a substituted or unsubstituted, straight chain or branched lower alkyl group.

Compounds of this invention in which W is an alpha aminoacyl group are illustrated by the following formula for members of the class in which Y and Z are trans C=C units:



wherein R' is -H or a substituted or unsubstituted, straight chain or branched, preferably lower, alkyl group. The -COCHR'NH2 moiety may be provided by any D- or L-amino acid, including but not limited to any of the naturally occurring amino acids.

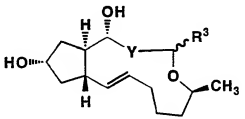
Another illustrative subclass of interest are compounds of the formula:



and the esters, amides and pharmaceutically acceptable salts thereof, including the individual diastereoisomers and their diastereomeric mixtures, wherein R" is H or a branched or straightchain, substituted or unsubstituted alkyl group, preferably lower alkyl. This subclass includes those compounds in which R" is a side-chain of an alpha amino acid, naturally occurring or otherwise. For instance,

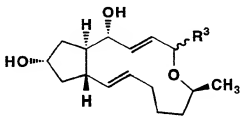
R" may be H (glycine), -CH_3 (alanine), -CH_2phenyl (phenylalanine), and so forth.

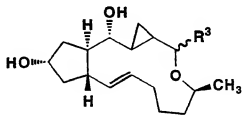
The corresponding ethers and acetals mentioned above are illustrated by compounds of the formula:



wherein $R^3$is -H, -OH, -alkoxy, -aryloxy, -arylalkoxy or -O-acyl and Y is a carbon-carbon double bond (trans) or a fused cyclopropyl group, as well as their esters and the individual diastereoisomers and their diastereomeric mixtures.

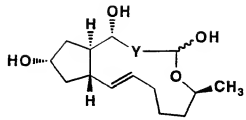
Several classes of such compounds are shown below:



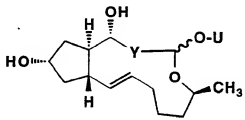


One group of compounds of interest are those in which R^3 is OH, i.e., hemiacetals. These hemiacetals are illustrated by the following structure, where Z in this case is a

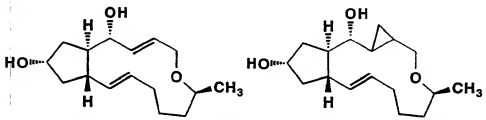
–C—C- unit and Y is as previously defined:



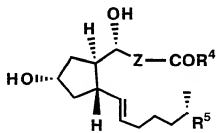
Another group of compounds of interest are those in which R^3 is-alkoxy, -aryloxy, -arylalkoxy or -O-acyl (i.e., -O-CO-U). These hemiacetals and derivatives have the following general structure, where Y is as previously defined and U is alkyl, aryl, aryloxy or acyl:



Another group of compounds of interest are the ethers (R^3= H), such as are illustrated in the following formulae in which Y is as previously defined and Z is a -C—C- unit:



Other related compounds include "open" chain compounds of the formula:

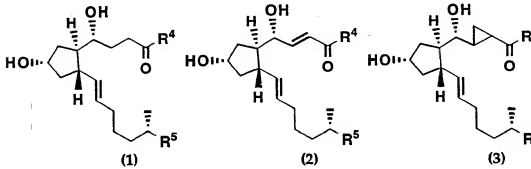


wherein R^4 is alkyl, alkenyl, aryl, arylalkyl, $-OR^6$ or NR^7R^8; where R^6 is alkyl, alkylene, aryl or arylalkyl and R^7 and R^8 are the same or different and are H, alkyl, alkylene, aryl or arylalkyl;

R^5 is H, OH, OR^9, $O(CO)R^9$, $O(CO)NR^{10}R^{11}$, $NR^{10}R^{11}$, =O, SO_2R^{10}, halo, trihalomethyl or $SiR^9R^{11}R^{12}$, where R^9, R^{12} and R^{13} may be the same or different and are alkyl, alkylene, aryl or arylalkyl; R^{10} and R^{11} may be the same or different and are H, alkyl, alkylene, aryl or arylalkyl; and,

Z is as previously described.

This invention thus encompasses three subclasses of compounds corresponding to formulas 1, 2 and 3:



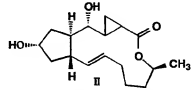
The compounds of this invention can be used as biological reagents to inhibit the intracellular transport of proteins from the ER of a cell through the compartments of the Golgi apparatus, and, ultimately, to the cell surface. For example, the compounds of this invention can be used to inhibit maturation of viral membrane glycoproteins in a cell infected with a virus by inhibiting the transport of viral membrane glycoproteins from the ER of the cell through the compartments of the Golgi apparatus, and, ultimately, to the cell surface, as required for the assembly of mature, infectious virus particles. These compounds can likewise be used to inhibit the transport, and thus the otherwise concomitant processing and presentation, of antigens by antigen presenting cells.

Compounds of this invention can also be used in pharmaceutical applications as antiviral, antifungal, immunosuppressive and antiproliferative agents (e.g., antitumor and anti-wart agents) and as detoxification agents. Accordingly, this invention further relates to pharmaceutical compositions which contain as active components compounds described herein which are effective for one or more of the indications noted herein and which can be administered to an individual in need thereof. For example, a compound of this invention can be combined with a physiologically compatible carrier for administration to an individual infected with a fungus or virus, or to an individual harboring a tumor. Without wishing to be bound by a particular theory, we do note that the compounds of this invention may act, at least in part, by inhibiting the transport of proteins critical, for example, to the maturation, intracellular replication and/or infectivity of virus, to the growth and/or proliferation of tumor cells, or to fungal growth.

SYNTHESIS

The compounds of this present invention can typically be produced synthetically from BFA. BFA can be prepared by fermentation followed by product recovery from the culture medium as described in detail in Harri, E., et al., Helv. Chim. Acta, 46:1235 (1963). Alternately, BFA can be synthesized using standard laboratory methods. (Baudouy, R., et al., Tetrahedron Letters, 34:2973-2976 (1977); LeDrian, C., et al., J. Am. Chem. Soc.,104:5473-5483 (1982); Kitahara, T. and Mori, K., Tetrahedron, 40:2935-2944 (1984)). BFA can then be transformed to produce the compounds described herein as described in the Examples which follow.

Another starting material is the cyclopropyl analog of BFA illustrated by formula (II):



II

which can be synthesized by reacting BFA with a slurry of trimethylsulfoxonium iodide, dimethyl sulfoxide and pentane-washed sodium hydride. An

illustrative procedure is provided in Example 4. The method of synthesis described herein results in a mixture of two diastereomers. Under the conditions described in the examples which follow, the 2R, 3R-diastereomer is the major product. The two diastereomers can be separated by standard laboratory methods. In addition, the apex of the cyclopropyl ring can be further modified to include one or two, halogens (e.g., fluoride), or, alternately, one, or two, unbranched alkyl groups. It should also be noted that the corresponding esters of the compounds disclosed herein (which esters are also encompassed by this invention and may be used for the purposes disclosed herein) can be prepared prior to or following final deprotection of the lactones. See e.g. USSN 08/207,319 and 08/207,496, *supra*..

Flouro and di-fluoro compounds of this invention can be prepared from the corresponding hydroxy (with stereochemical inversion) and keto compounds, respectively, (typically with protection of other hydroxy groups and subsequent deprotection) using diethylaminosulfur trifluoride (DAST). See e.g. Middleton et al, J Fluorine Chem (1983) 23:557 (conversion of hydroxyl to fluoro); Middleton et al, J Fluorine Chem (19800 45:2883 (conversion of keo to difluoro); and M. Hudlicky, Organic Reactions (1988), vol 35, pp. 513 et seq (review).

EVALUATION OF *IN VITRO* BIOLOGICAL ACTIVITY

The biological activity of these compounds can be evaluated and compared using conventional *in vitro* assays for inhibition of protein trafficking between the endoplasmic reticulum (ER) and the Golgi apparatus and specifically for antiviral, antitumor, immunosuppressive and antifungal activity as discussed in further detail below.

(a) Inhibition of Protein Transport

The inhibitory activity of the compounds with respect to protein transport can be evaluated in a cell-free system as described in Orci, L., et al., Cell, 64:1183-1195 (1991). Generally, secretory proteins, such as membrane glycoproteins, are transported from the endoplasmic reticulum to the Golgi apparatus, and subsequently to the cell surface, via transport vesicles. To evaluate the ability of the compounds described herein to prevent the formation of transport vesicles, Golgi apparatus membranes can be isolated and incubated with cytosol, ATP, an

ATP regenerating system and the compounds to be tested as described in Orci, L., et al., Cell, 64:1183-1195 (1991).

The activity of our compounds in inhibiting protein transport may also be evaluated using a Guanine Nucleotide Exchange Factor (GEF) assay as described in detail in the Examples. The GEF assay is based on assays described in Donaldson, J. G., et al., Nature, 360:350-352 (1992), and Helms, J. B., et al., Nature, 360:352-354 (1992). A number of cytosolic proteins are specifically associated with the Golgi apparatus. One such protein, β-COP, is rapidly released from the Golgi upon treatment with BFA. This release occurs within 20 seconds of BFA treatment and is complete in 1-2 minutes. Upon removal of BFA, β-COP rapidly reassociates with the Golgi apparatus. (Klausner, R.D., et al., J. Cell Biol. 116:1071-1080 (1992)). The binding of β-COP to Golgi membranes has been shown to be dependent on the interaction of another protein, ADP-ribosylation factor (ARF) with the Golgi membrane. ARF association with the Golgi is, in turn, dependent on binding the guanine nucleotide, GTP. A component of Golgi membranes specifically catalyzes the exchange of GTP onto ARF. BFA prevents the assembly of β-COP onto the Golgi membrane by inhibiting the GTP-dependent interaction of ARF with the Golgi membrane. (Donaldson, J. G., et al., Nature, 360:350-352 (1992); Helms, J. B., et al., Nature, 360:352-354 (1992)).

The activity of our compounds in preventing Golgi membranes from catalyzing the exchange of GTP onto ARF may be evaluated as described in the Examples below.

(b) Anti-viral Activity

BFA has been shown to have dramatic effects on membrane protein glycosylation and processing, key steps which affect the egress of enveloped viruses from infected cells. (Whealy, M. E., et al., J. Virol., 65:1066-1081 (1991)). The envelopment of a virus, during the maturation process in an infected host cell, appears to be a multistep pathway. The viral capsid acquires a membrane by budding of the capsid through the nuclear membrane such that an immature enveloped virion is formed. This immature virion is transported through the endoplasmic reticulum (ER) and undergoes subsequent de-envelopment, with release of the immature virus particle in proximity to the Golgi apparatus. Subsequent maturation of the immature virion occurs at the Golgi apparatus, which involves a second envelopment of these immature capsids by membrane

proteins derived from the Golgi apparatus, containing fully processed viral glycoproteins. The resulting mature, infectious enveloped virus particle is released from the cell by fusion of the outer membrane of the virion envelope with the plasma membrane of the host cell, or, alternately, can be transported via transport vesicles to the cell surface, where membrane fusion results in presentation of viral glycoproteins on the cell surface. BFA does not affect protein synthesis at the translational level, but blocks the post-translational processing and export of viral glycoproteins to the Golgi apparatus, thus, inhibiting viral replication by preventing the formation and/or release of mature, infectious virus particles.

The compounds of this invention can be tested for specific antiviral activity as described in Example 7 as well as by other conventional antiviral assay methods. See e.g. Whealey et al, supra; Johnson et al, J Virol 43(3):1102-1112 (1982); Sidwell et al, Nucleotides and Nucleosides 8:833-836 (1989) and Chen et al, J Virol 65(3):1427-1439 (1991). As described in detail in Example 7 the antiviral activity and non-specific cytotoxic effects of these compounds can be readily evaluated using Hep2 cells infected with Herpes Simplex Virus type 1 (HSV-1). BFA can be used as a control, as can clinically relevant or other known positives, such as IUDR (iodouracyl deoxyribocyte) which can be used as an antiviral, positive control.

(c) Evaluation of Other *in vitro* Activities

Compounds can be evaluated with respect to specific antifungal, anticancer, immunosuppressive or other pharmaceutically relevant activities using conventional materials and methods. See e.g. Arioka, J. Gen. Microbiol., 137:1253-1262 (1991) (evaluation of antifungal activity); Ishii et al., J. Antibiot. XLII:1877-1878 (1989) (evaluation of cytotoxic/antitumor activity); Sun et al, US Patent 5,206,249 (27 April 1993)(evaluation of *in vitro* growth inhibitory activity on cultured leukemia cells); and Yoshida et al., Experimental Cell Research 192:389-395 (1991)(evaluation of anti-toxin activity).

EVALUATION OF *IN VIVO* BIOLOGICAL ACTIVITY

Bioactivity can be further evaluated in conventional animal model systems including anti-viral, anti-fungal, antitumor, immunosuppression and detoxification assays involving experimental animal models, e.g. using rats, mice,

rabbits, guinea pigs, sheep or non-human primates. Numerous animal models for such studies, as well as animal models for determining biological half-life, pharmacokinetics and toxicology, are well known in the art. *In vivo* toxicity can be readily evaluated with conventional toxicity assays as well as by the method described in Example 8.

(a) *in vivo* antiviral activity

The effectiveness of the compounds of this invention in controlling viral infection can be evaluated in any of the conventional assay systems. See e.g. Stanberry, "Pathogenesis of Herpes Simplex Virus Infection and Animal Models for its Study" and Renegar, Laboratory Animal Science 42(3):222. For instance, HSV infection can be evaluated using guinea pig and mouse model systems that are art-recognized models used in the study of genital herpes. The guinea pig model system is described in detail in Stanberry, L.R., et al., J. Infect. Diseases, 153:1055-1061 (1986), and Bourne, N., et al., Antimicrob. Agents and Chemo., 36:2020-2024 (1992). The effectiveness of antiviral agents against influenza virus can be evaluated in mice as described by Sidwell et al, in Antiviral Res. 6:343-353 (1985) and in Antimicrob. Ag. Chemother. 36:473-476 (1992).

(b) *in vivo* antitumor activity

The antitumor effectiveness of our compounds can be evaluated *in vivo* with conventional xenograft models using various human tumor cell lines xenografted into mice as described, for example, in Sun et al, supra, as well as in various transgenic animal models (again, see Sun et al, col 21).

PHARMACEUTICAL APPLICATIONS

Compounds of this invention which prevent, inhibit or reduce the severity of viral infection (e.g. an infection by a virus such as a Herpes Simplex virus), fungal infection (e.g. an infection by a fungus such as Candida albicans), tumors or tumor growth or the effect of toxic substances or which have an immunosuppressive effect may be used in pharmaceutical compositions and methods for treatment or prevention in a mammal in need thereof.

Mammals include rodents such as mice, rats and guinea pigs as well as dogs, cats, horses, cattle, sheep, non-human primates and humans.

The preferred method of such treatment or prevention is by administering to a mammal an effective amount of a compound of this invention to prevent, alleviate or cure said disease or disorder. An effective amount of a compound of this invention is an amount of one or more compounds of this invention which inhibits one or more of protein transport from the endoplasmic reticulum, viral replication, fungal growth, tumor cell growth and pathological effect(s) of a toxin, or which results in immunosuppression, as the case may be. Such effective amounts can be readily determined by evaluating the compounds of this invention in conventional assays well-known in the art, including assays described herein.

Therapeutic/Prophylactic Administration & Pharmaceutical Compositions

The invention provides methods of treating, preventing and/or alleviating the symptoms and/or severity of a disease or disorder referred to above by administration to a subject a compound of the invention in an amount effective therefor. The subject will be an animal, including but not limited to animals such as cows, pigs, chickens, etc., and is preferably a mammal, and most preferably human.

Various delivery systems are known and can be used to administer a compound of this invention, e.g., encapsulation in liposomes, microparticles, microcapsules, etc. One mode of delivery of interest is via pulmonary administration, as detailed more fully infra. Other methods of introduction include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural and oral routes. A compound of this inventions may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. For treatment or prophylaxis of nasal, bronchial or pulmonary infections or tumors, preferred routes of administration are oral, nasal or via a bronchial aerosol or nebulizer.

In specific embodiments, it may thus be desirable to administer a compound of this invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application (e.g., for viral or fungal infections or tumors of the

skin), by injection, by means of a catheter, by means of a suppository, or by means of a skin patch or implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers.

This invention also provides pharmaceutical compositions. Such compositions comprise a therapeutically (or prophylactically) effective amount of a compound of this invention, and a pharmaceutically acceptable carrier or excipient. Such a carrier includes but is not limited to saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The carrier and composition can be sterile. The formulation should suit the mode of administration.

The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. The composition can be a liquid solution, suspension, emulsion, tablet, pill, capsule, sustained release formulation, or powder. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc.

In a specific embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic to ease pain at the side of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

Administration to an individual of an effective amount of one or more of the compounds described herein can also be accomplished topically by

administering the compound(s) directly to the affected area of the skin of the individual. For this purpose, the compounds are administered or applied in a composition including a pharmacologically acceptable topical carrier, such as a gel, an ointment, a lotion, or a cream, which includes, without limitation, such carriers as water, glycerol, alcohol, propylene glycol, fatty alcohols, triglycerides, fatty acid esters, or mineral oils.

Other topical carriers include liquid petroleum, isopropyl palmitate, polyethylene glycol, ethanol (95%), polyoxyethylene monolaurate (5%) in water, or sodium lauryl sulfate (5%) in water. Other materials such as anti-oxidants, humectants, viscosity stabilizers, and similar agents may be added as necessary.

In addition, in certain instances, it is expected that the compounds of this invention may be disposed within devices placed upon, in, or under the skin. Such devices include patches, implants, and injections which release the compound into the skin, by either passive or active release mechanisms.

In a specific application of this invention, we note that genital infection with HSV is characterized by herpetic lesions on the external genital skin. As a consequence of initial genital infection, latent infection is established. One possible mechanism for the maintenance of latency involves the migration of virus from recurrent lesions back to sensory ganglia, where a new set of neurons are infected and become a source of latent virus responsible for recurrent disease. (Stanberry, L.R., et al., J. Infect. Dis., 153:1055-1061 (1986)). Thus, administration of an antiviral agent which inhibits the formation of mature infectious virus particles would be useful to prevent migration of HSV and reasonably prevent establishment of a latent HSV infection. Topical administration of a compound of this invention directly to the areas of the skin affected with the herpetic lesions would be an attractive method of administration. As an illustrative example of anti-viral application of a pharmaceutical agent, see Whitley et al, "Acyclovir: A Decade Later", New England Journal of Medicine pp. 782-789 (10 September 1992).

Materials and methods for producing the various formulations are well known in the art [see e.g. US Patent Nos. 5,182,293 and 4,837,311 (tablets, capsules and other oral formulations as well as intravenous formulations)].

A compound of this invention can be formulated in neutral or salt forms. Pharmaceutically acceptable salts include those formed with free amino

groups such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with free carboxyl groups such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2- ethylamino ethanol, histidine, procaine, etc.

The effective dose of compounds of this invention will typically be in the range of about 0.01 to about 50 mg/kgs, preferably about 0.1 to about 10 mg/kg of mammalian body weight, administered in single or multiple doses. Generally, the compounds of this invention may be administered to patients in need of such treatment in a daily dose range of about 1 to about 2000 mg per patient.

The amount of a compound of this invention which will be effective in the treatment or prevention of a particular disorder or condition will depend on the nature of the disorder or condition, and can be determined by standard clinical techniques. In addition, *in vitro* or *in vivo* assays may optionally be employed to help identify optimal dosage ranges. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems. The precise dosage level of our compounds, as the active component(s), should be determined by the attending physician or other health care provider and will depend upon well known factors, including route of administration, biological activity of the particular compound, and the age, body weight, sex and general health of the individual; the nature, severity and clinical stage of the disease; and the use (or not) of concomitant therapies.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceutical or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

Pulmonary Administration

In an embodiment of this invention of particular interest, a compound of this invention is administered by pulmonary administration, e.g. via aerosolization. This route of administration may be particularly useful for treatment or prophylaxis of bronchial or pulmonary infection or tumors.

Pulmonary administration can be accomplished, for example, using any of various delivery devices known in the art (see e.g., Newman, S.P., 1984, in Aerosols and the Lung , Clarke and Davia (eds.), Butterwarths, London, England, pp. 197-224; PCT Publication No. WO 92/16192 dated October 1, 1992; PCT Publication No. WO 91/08760 dated June 27, 1991; NTIS Patent Application 7-504-047 filed April 3, 1990 by Roosdorp and Crystal), including but not limited to nebulizers, metered dose inhalers, and powder inhalers. Various delivery devices are commercially available and can be employed, e.g., Ultravent nebulizer (Mallinckrodt, Inc., St. Louis, Missouri); Acorn II nebulizer (Marquest Medical Products, Englewood, Colorado), Ventolin metered dose inhaler (Glaxo Inc., Research Triangle Park, North Carolina); Spinhaler powder inhaler (Fisons Corp., Bedford, Massachusetts) or Turbohaler (Astra). Such devices typically entail the use of formulations suitable for dispensing from such a device, in which a propellant material may be present.

Ultrasonic nebulizers tend to be more efficient than jet nebulizers in producing an aerosol of respirable size from a liquid (Smith and Spino, "Pharmacokinetics of Drugs in Cystic Fibrosis," Consensus Conference, Clinical Outcomes for Evaluation of New CF Therapies, Rockville, Maryland, December 10-11, 1992, Cystic Fibrosis Foundation).

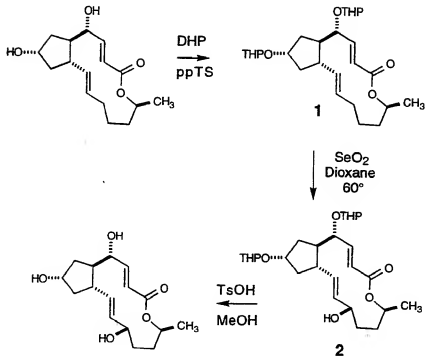
A nebulizer may be used to produce aerosol particles, or any of various physiologically acceptable inert gases may be used as an aerosolizing agent. Other components such as physiologically acceptable surfactants (e.g., glycerides), excipients (e.g., lactose), carriers, and diluents may also be included.

This invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description. Such modifications are intended to fall within the the scope of the appended claims.

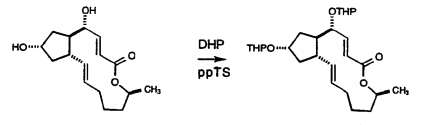
Various patents, patent applications and publications are cited herein, the disclosures of which are incorporated by reference in their entireties.

EXAMPLES

Example 1. Preparation of (12R)-OH BFA by the following route:

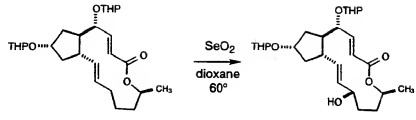


A. Di-THP BFA



To a magnetically stirred solution of brefeldin A (2.0 g, 7.1 mmol) in 100 mL CH_2Cl_2 was added 3,4-dihydro-2*H*-pyran (2.8 mL, 30.7 mmol) and pyridinium toluene-*p*-sulphonate (10 mg, 0.04 mmol). The reaction was stirred at room temperature for 16 h, then diluted with CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated to afford 3.2 g (quantitative yield) of bis-tetrahydropyranyl protected brefeldin A **1**. Compound **1** was judged sufficiently pure by ^1H NMR analysis to be used in the subsequent reaction without further purification.

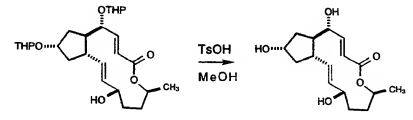
B. Hydroxylation



1 2

To a magnetically stirred solution of protected brefeldin A **1** (3.2 g, 7.0 mmol) in 60 mL of 1,4 dioxane was added selenium dioxide (1.6 g, 14 mmol). The mixture was warmed to 60 °C and stirred at this temperature for 48 h. The reaction was allowed to cool to room temperature and stirred another 24 h. The suspension was filtered through celite, washing with EtOAc. The filtrate was diluted with more EtOAc and washed with saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated. The crude product was purified via flash chromatography (50% ethyl acetate in hexanes) to provide 2.4 g (73% yield) of alcohol **2** as a white solid. ^1H spectra of **2** agreed with the proposed structure.

C. Deprotection

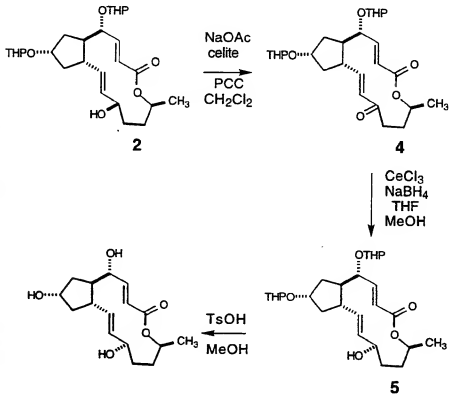


2

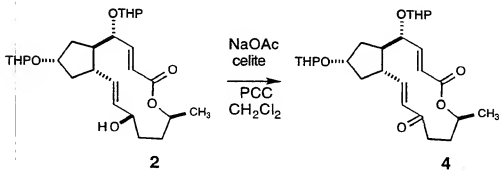
To a magnetically stirred solution of alcohol **2** (800 mg, 1.7 mmol) in 20 mL of anhydrous MeOH was added *p*-toluenesulfonic acid monohydrate (4 mg, 0.02 mmol) and stirred at room temperature for 5 h. The reaction was quenched with solid $NaHCO_3$, filtered, and concentrated. The crude product was purified via flash chromatography (1% methanol in ethyl acetate) to provide 430 mg (84%

yield) of a white solid. Single-crystal X-ray analysis of the product independently confirmed the structure as (12*R*)-OH brefeldin A; HRMS (FAB, negative ion) calcd for $C_{16}H_{23}O_5$: 295.1545, found 295.1556.

Example 2: Preparation of (12*S*)-OH Brefeldin A by the following route:

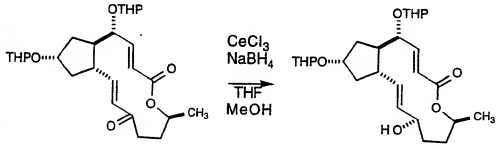


A. Oxidation



To a magnetically stirred solution of alcohol 2 (1.2 g, 2.6 mmol) in 26 mL of CH_2Cl_2 was added sodium acetate (320 mg, 3.9 mmol), celite (900 mg), and pyridinium chlorochromate (840 mg, 3.9 mmol). The reaction was stirred at room temperature for 4 h, then filtered through a bed of celite rinsing the solids with CH_2Cl_2 before concentrating. The crude product was purified via flash chromatography (50% ethyl acetate in hexanes) to provide 960 mg (81% yield) of ketone 4. ^1H spectra of 4 agreed with the proposed structure.

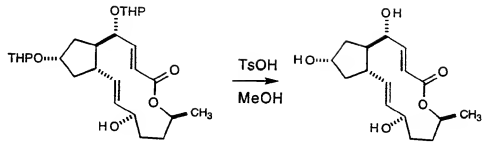
B. Stereospecific reduction



4 5

To a magnetically stirred solution of ketone 4 (400 mg, 0.87 mmol) in 5 mL of THF and 12 mL of MeOH was added cerium (III) chloride (210 mg, 0.87 mmol) and stirred at room temperature for 25 min. The mixture was cooled to 0 °C and sodium borohydride (33 mg, 0.87 mmol) added. The reaction was stirred at 0 °C for another 5 min then warmed to room temperature and stirred for 20 min. The reaction was quenched with a 1:1 mixture of saturated aqueous NH_4Cl and H_2O and concentrated to remove the organic solvents. The aqueous residue was extracted with three portions of EtOAc. The combined organic extracts were dried over $MgSO_4$, filtered, and concentrated to afford 400 mg (quantitative yield) of alcohol 5. Compound 5 was judged sufficiently pure by ^1H NMR analysis to be used in the subsequent reaction without further purification.

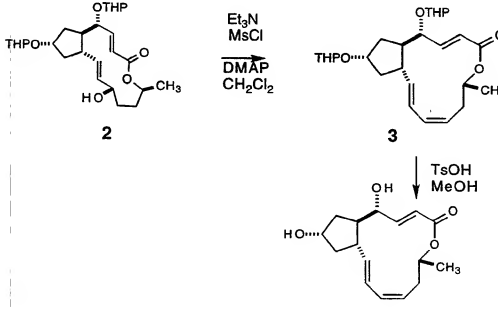
C. Deprotection



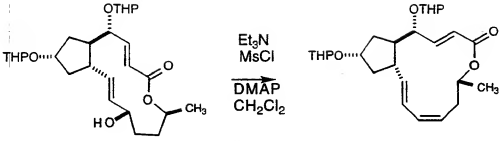
5

To a magnetically stirred solution of alcohol 5 (310 mg, 0.67 mmol) in 7 mL of anhydrous MeOH was added *p*-toluenesulfonic acid monohydrate (1 mg, 0.007 mmol) and stirred at room temperature for 5 h. The reaction was quenched with solid $NaHCO_3$, filtered, and concentrated. The crude product was purified via flash chromatography (1% methanol in ethyl acetate) to provide 170 mg (85% yield) of a white solid. Single-crystal X-ray analysis of the product independently confirmed the structure as (12*S*)-OH brefeldin A; HRMS (FAB, negative ion) calcd for $C_{16}H_{23}O_5$: 295.1545, found 295.1538.

Example 3: Preparation of 12,13 Dehydro-Brefeldin A by the following route:



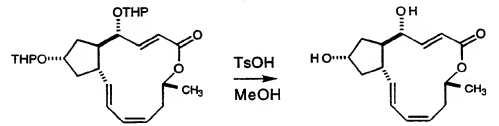
A. Elimination



2 3

To a magnetically stirred solution of alcohol 2 (740 mg, 1.6 mmol) in 16 mL of CH_2CL_2 at 0 °C was added triethylamine (490 µL, 3.5 mmol), 4-dimethylamino-pyridine (2.0 mg, 0.016 mmol), and methanesulfonyl chloride (190 µL, 2.4 mmol). The mixture was stirred at 0 °C for 1 h then warmed to room temperature and stirred another 30 min. The reaction was diluted with CH_2CL_2 and washed with saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated. The crude product was purified via flash chromatography (30% ethyl acetate in hexanes) to provide 630 mg (89% yield) of triene 3 as a white foam. ^1H spectra of 3 agreed with the proposed structure.

B. Deprotection

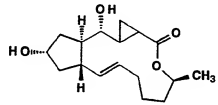


3

To a magnetically stirred solution of triene 3 (600 mg, 1.4 mmol) in 13 mL of anhydrous MeOH was added *p*-toluenesulfonic acid monohydrate (3 mg, 0.014 mmol) and stirred at room temperature for 5 h. The reaction was quenched with solid $NaHCO_3$, filtered, and concentrated. The crude product was purified via flash chromatography (80% ethyl acetate in hexanes) to provide 220 mg (58% yield) of a white solid. Single-crystal X-ray analysis of the product

independently confirmed the structure as 12,13-Dehydro-brefeldin A; HRMS (FAB, negative ion) calcd for $C_{16}H_{22}O_4$: 277.1440, found 277.1440.

Example 4: Synthesis of Cyclopropyl derivative of BFA **(II)**



II

Trimethylsulfoxonium iodide (0.258 gram) was added to a 10 mL flask containing a stir bar, nitrogen inlet, 2.5 mL dimethyl sulfoxide (DMSO) and pentane washed NaH. The resulting slurry was stirred at room temperature until gas evolution ceased (15 minutes). Brefeldin A (0.107 gram) was added at once and stirring was continued for an additional hour at ambient temperature. The reaction mixture was quenched with water (4 mL) and extracted with ethyl acetate (4x 50 mL). The combined organic extract was washed with water (2x 25 mL), brine (25 mL) and dried over sodium sulfate. The solvent was removed under vacuum and the residue chromatographed through a silica gel column, eluted with ethyl acetate, to separate isomers.

The major R, R-isomer gave an oil which crystallized upon trituration with acetonitrile. Recrystallization from acetonitrile gave crystals suitable for x-ray crystallography. The structure was consistent with the R, R-isomer as determined by x-ray crystallography and the following $^1H/^{13}C$ NMR data:

^1H NMR ($CDC1_3$)ppm 1.0 (d,2H, Me) 1.1-1.65(m, 8H) 1.7-2.25(m,10H) 3.5(d,1H,C(4)H-OH) 4.15(m,1H, C(7)H-OH) 4.9(m,1H, C(15)H-OR) 5.3 (m,2H, C(10)H=C(11)H)

^{13}C NMR ($CDC1_3$) ppm 173.9, 137.1, 129.4, 73.1, 72.6, 69.6, 50.4, 44.9, 44.1, 41.1, 34.2, 30.1, 26.2, 25.1, 20.6, 15.9, 11.1.

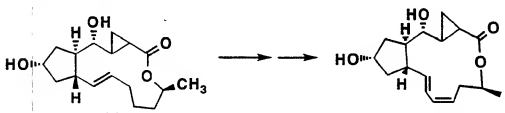
The minor S,S-isomer was isolated as an oil. The S,S-isomer structure was consistent with the following $^1H/^{13}C$ NMR data:

^{1}H NMR ($CDC1_3$) ppm 0.75(m,1H) 1.08(d, 2H, CH_3) 1.1-1.2(m,1H) 1.25-1.4(m, 5H) 1.55(m, 1H) 1.7(m, 1H) 1.8-2.2(m, 8H) 2.3(m, 1H) 3.7(m, 1H) 4.2(m, 1H) 4.8(d, 1H) 5.3(m, 2H)

^{13}C NMR ($CDC1_3$) ppm 12.5, 18.0, 22.0, 23.7, 25.7, 32.5, 35.7, 38.9, 42.9, 43.7, 49.2, 68.0, 71.8, 81.5, 131.4, 133.6, 176.8.

These compounds may be used in place of BFA in the syntheses decribed herein.

Example 5. Preparation of Cyclpropyl derivatives



Examples 1, 2 and 3 may be carried out using Compound II (R,R, S,S or a mixture of the two) in place of BFA to yield the corresponding 12-hydroxy and triene compounds bearing a 2,3 cyclopropyl group.

Example 6: Guanine Nucleotide Exchange Factor Assay

Recombinant myristoylated ADP-ribosylation factor (ARF) is purified from Escherichia coli co-expressing the human ARF-1 gene and N-myristoyltransferase as described in Weiss, O., et al., J. Biol. Chem., 264:21066-21072 (1989) and Duronio, R. J., et al., Proc. Natl. Acad. Sci. USA, 87:1506-1510 (1990).

Golgi membranes from rat livers are obtained by sucrose gradient centrifugation as described in Balch, W. E., et al., Cell, 39:525-536 (1984).

Incubations are carried out as described in Donaldson, J.G., et al., Nature 360:350-352 (1992) and Helms, J.B., and Rothman, J.E., Nature 360:352-354 (1992). Briefly, a 50.5 µl reaction mixture containing ARF, Golgi membranes, sucrose, ovalbumin, HEPES-KOH buffer containing KCI and Mg, 100 µM compound and [^{35}S]GTP is incubated at 37°C for 15 minutes. The specific reactions are set up

with 5µl of 2.3M sucrose, 10 µl of 0.5 mM compound (except for the background and control reactions, in which no compound is added), 5 µl buffer, 5 µl of 16 mg/ml Ovalbumin, 5 µl of 0.6 mg/ml golgi (none in background run), 8 µl of 0.4 gm/ml Arf-1 (except none in background run) and 12.5 µl of 20 µM GTP (^{35}S), with the background and control reactions diluted with 23 µl and 10 µl, respectively, of water.

The amount of ARF-bound and -unbound [^{35}S]GTP is separated with 10 kD molecular weight cutoff cellulose filters. Nonspecific binding (from background run) is subtracted.

Alternatively, the ARF-bound [^{35}S]GTP can be separated by Sephadex G25 gel filtration.

Example 7: Test for Antiviral Activity

The antiviral activity and cytotoxic microscopic effects of our compounds may be determined in the following manner.

Hep2 cells in RPMI/1640 medium with 5% fetal calf serum are grown to provide a confluent sheet of cells. Various concentrations of the compound(s) to be tested, as well as positive and negative controls, are added. The cells are then incubated at 37°C, in 5% carbon dioxide.

In a cytotoxicity screening assay, different concentrations of the compound(s) to be tested, as well as positive and negative controls, are added to the cell culture. Cytotoxicity is determined by microscopic examination on days 3 and 6.

The antiviral and cytotoxic effects of the compounds on Hep2 cells infected with HSV-1 can be determined as follows.

Hep2 cells in RPMI/1640 medium with 5% fetal calf serum are added to microtiter wells and incubated at 37°C in 5% carbon dioxide. To the Hep2 cells, various concentrations (4 wells/concentration compound) of the compound(s) to be tested, as well as positive and negative controls, are added. Typical test concentrations may run from about 0.1-50 µg/ml. HSV-1 virus is added 7 hours later. The cell culture specimens are examined for evidence of viral growth and Hep2 microscopic toxicity on days 1, 2, 3, 4, 5, 6, 7 and 10 post-infection. Virus controls at dilutions of 10^{-10} to 10^{-14} are included.

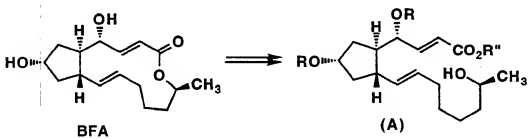
Example 8: *In vivo* Toxicity Study

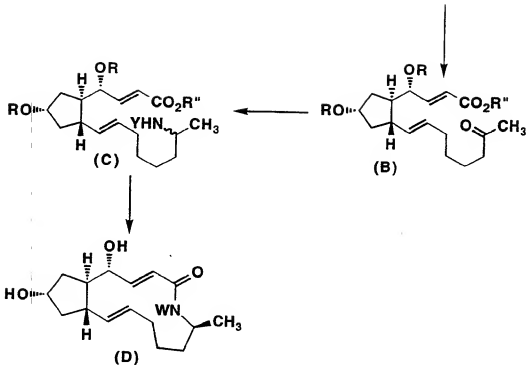
An intraperitoneal dose response study of the compounds of this invention can be carried out in mice to provide toxicological data as follows. Female CD-1 mice (6/group) are employed in this investigation. The compound is suspended in 0.5% carboxymethylcellulose (CMC). The animals are scheduled to receive the compound, intraperitoneally, at dosage levels of 50, 100 and 400 mg/kg/day for 5 consecutive days. All doses are administered in a constant volume of 20 ml/kg. Another group of animals receives 0.5% CMC (20 ml/kg) and serves as a control. The animals are then observed for at least two weeks after which a necropsy is performed. Tissues are collected for histopathologic evaluation.

Example 9: Additional Synthetic Examples

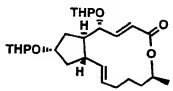
A. Lactams

The compounds of this present invention can be produced synthetically from BFA or other starting materials. BFA can be prepared by fermentation followed by product recovery from the culture medium as described in detail in Harri, E., et al., Helv. Chim. Acta, 46:1235 (1963). Alternately, BFA can be synthesized using standard laboratory methods. (Baudouy, R., et al., Tetrahedron Letters, 34:2973-2976 (1977); LeDrian, C., et al., J. Am. Chem. Soc.,104:5473-5483 (1982); Kitahara, T. and Mori, K., Tetrahedron, 40:2935-2944 (1984)). BFA can then be transformed to produce the lactams described herein by the following general strategy:





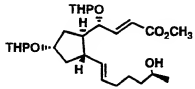
Example A-1. 4,7 Ditetrahydropyranyl Brefeldin A **(A-I)**



(A-I)

To a stirring suspension of Brefeldin A (1.0 gm) in methylene chloride (50 mL) at room temperature is added dihydropyran (1.2 gm) followed by pyridinium p-tosylate (ppts) (40 mgm). After the suspension clears, stirring is continued for an additional hour. The solution is then diluted with methylene chloride and washed successively with water, saturated sodium bicarbonate and brine. The organic layer is dried over sodium sulfate, filtered and concentrated under reduced pressure to a constant weight.

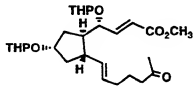
Example A-2. γ,4 dihydroxy-2-[6-hydroxy-1-heptenyl]-4-cyclopentane methyl crotonate **(A-II)**



(A-II)

N-butyl lithium (2.5N, 2.75 mL) is slowly added to stirring, anhydrous methanol (8 mL) cooled in a -20°C bath. After 10-30 minutes, the -20°C bath is removed and the solution allowed to come to room temperature. A methanolic solution of compound I is added and stirring is continued for 5 hours at room temperature. The solution is concentrated under reduced pressure and the residue taken up in ethyl acetate (50 mL) and water (20 mL). The organic layer is washed successively with saturated sodium bicarbonate water and brine. The organic layer is dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue may then be column chromatographed over silica gel (ethyl acetate/ hexane (1:4)).

Example A-3. γ,4 Ditetrahydropyranylhydroxy-2-[6-oxo-heptenyl]-4-cyclopentane methylcrotonate **(A-III)**

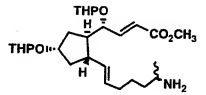


(A-III)

To a stirring suspension of pyridinium chlorochromate (PCC) (1.15 gm) and sodium acetate (440 mgm) in dry methylene chloride under an N_2 atmosphere at room temperature, is immediately added compound A-II (1.70 gm) in methylene chloride (2 mL). The resulting suspension is stirred at room temperature for 5 hours, when no starting material is visible by TLC (hexane/ ethyl acetate 1:1). The suspension is poured into stirring ethyl ether (150 mL)

and stirred for an additional 15 minutes. The resulting slurry is filtered, and concentrated. The residue may be purified by column chromatography if desired on silica gel (hexane/ ethyl acetate (3:1).

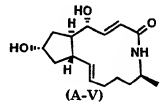
Example A-4. γ, 4 ditetrahydropyranylhydroxy-2-[6-amino-heptenyl]-4-cyclopentane methylcrotonate **(A-IV)**



(A-IV)

To a solution of acetic acid (0.24 mL) and ammonia (0.35 gm) in anhydrous methanol (12 mL) stirring under a N_2 atmosphere in a 0°C bath, is added compound A-III (1.0 gm) in THF (10 mL). The resulting mixture is stirred for 2 hours in the 0°C bath, after which sodium cyanoborohydride (0.20 gm) in anhydrous methanol (0.5 mL) is slowly added over 5 minutes. After 4 hours at 0°C, the temperature is allowed to rise to room temperature and stirring continued for an additional 36 hours. The reaction solution is then poured into ethyl acetate (100 mL) and extracted sequentially with water, saturated sodium bicarbonate and brine. The organic layer is dried over sodium sulfate, filtered and concentrated. This residue may be purified if desired by column chromatography over silica gel (hexane/ETAc, 2:1).

Example A-5. Lactam **(A-V)**

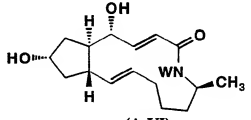


(A-V)

To a stirred solution of **A-IV** (1.0 gm) in methylene chloride (10 mL) under N_2, cooled to 0°C, is added by slow addition over 10 minutes trimethyl aluminum (1 mL) in hexane (2.5 N). After 10 minutes the reaction is brought to

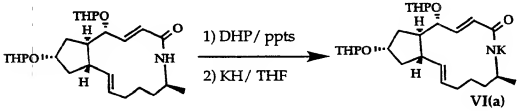
reflux for 48 hr. The solution is cooled to room temperature, and hydrolyzed by the slow addition of 0.1 N HCl (21 mL). The mixture is stirred for an additional 30 minutes and diluted with ethyl acetate. The organic layer is separated and extracted sequentially with water, saturated sodium bicarbonate and brine. The organic layer is dried over sodium sulfate, filtered and concentrated. The residue is then taken up in anhydrous methanol (20 mL), treated with toluene sulfonic acid (40 mgm) and vigorously stirred at room temperature for 6 hr. The solvent is remove under reduced pressure, and the residue taken into ethyl acetate and sequentially extracted with water, saturated sodium bicarbonate and brine. The organic layer was dried over sodium sulfate, filtered and concentrated.

Example A-6. Substituted lactams **(A-VI)**

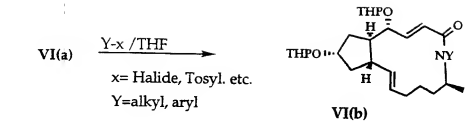


(A-VI)

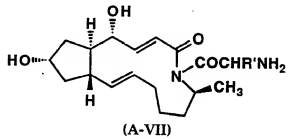
Lactams of the formula (A-VI) where W represents an alkyl group may be prepared from A-III by adaptation of the method of Example A-4 but substituting the desired amine for ammonia. Alternatively, such compounds may be prepared by the following route followed by deprotection:



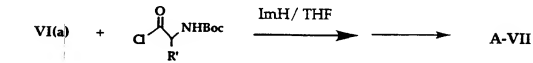
VI(a)



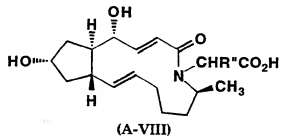
Example A-7. Substituted lactams **(A-VII)**



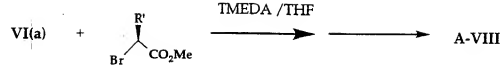
Substituted lactams of the formula A-VII may be prepared by the following route using conventional transformation and deprotection methods:



Example A-8. Substituted lactams **(A-VIII)**

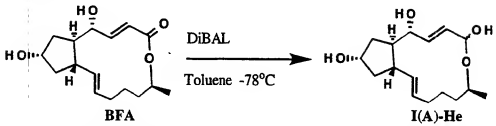


Substituted lactams of the formula A-VIII may be prepared by the following route using conventional transformation and deprotection methods:



B. Acetals, Hemiacetals and ethers

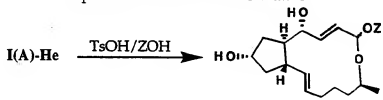
Example B-1. Preparation of hemiacetals



BFA **I(A)-He**

Brefeldin A may be converted to the hemiacetal with DiBAL by conventional methods. See e.g. J Org Chem 47, 4750 (1982) and J Med Chem 28, 1580 (1985).

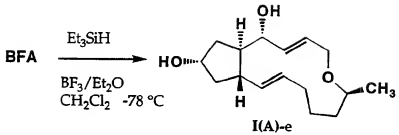
Example B-2. Preparation of acetals and derivatives



I(A)-Ac

Compounds of the formula (IA)-Ac, where Z represents an alkyl, aryl, arylalkyl or acyl group, may be prepared from IA-He using conventional methods and materials. See e.g. Baker, R. et al, J Chem Soc PT I 47, 1990.

Example B-3. Preparation of ethers



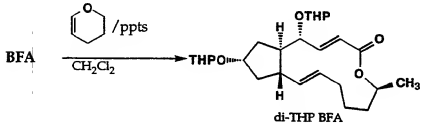
I(A)-e

I(A)-e may be prepared from BFA by adaptation of the method of Kraus, G.A. et al, J Org Chem 55, 1105 (1990) and J Org Chem 45, 4820 (1980).

C. Open chain compounds

Example C-1. Preparation of Compounds of Formula **(C-2)**

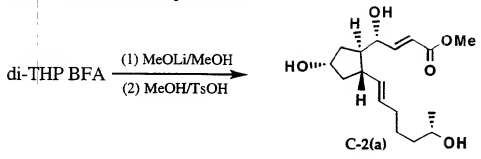
(A) Synthesis of di-THP BFA



di-THP BFA

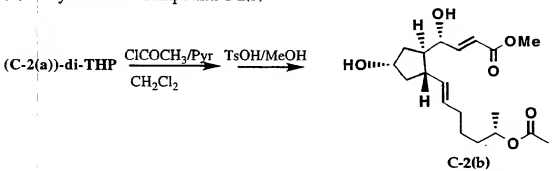
THP protecting groups may be added to BFA as described in J Org Chem 44, 1439 (1979)

(B) Synthesis of Compound C-2(a)

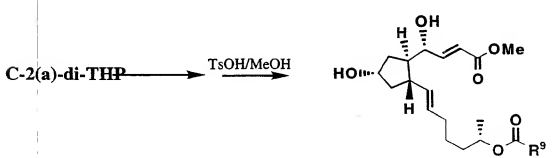


The di-THP BFA lactone may be converted into the methyl ester of the open-chain alcohol using methyl lithium as described in JCS Chem Comm 695, 1986, followed by removal of the THP groups with MeOH/TsOH. Other esters may be produced by conventional methods such as ester exchange using organolithium reagents containing the desired OR^4 group, as exemplified below.

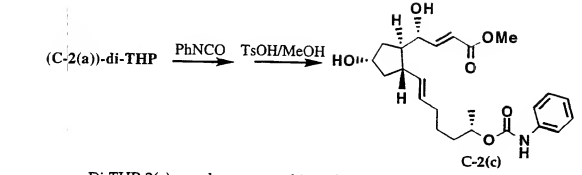
(C) **Synthesis of Compound C-2(b)**



Di-THP-(C-2(a)) may be readily acetylated (e.g., J Am Chem Soc 100, 8272 , 1978) and deprotected to yield C-2(b). Di-THP-(C-2(a)) may alternatively be acylated with any desired -(CO)R^9 moiety using any number of conventional coupling approaches and then deprotected:

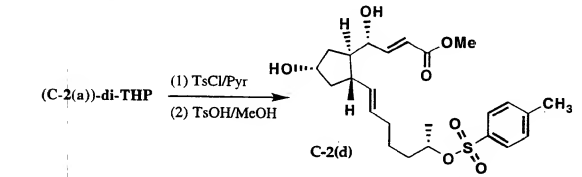


(D) Synthesis of Compound C-2(c)



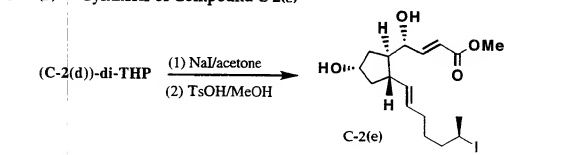
Di-THP-2(a) may be converted into the desired urethane using conventional procedures such as condensation of the alcohol with the appropriate isocyanate (see e.g. J Org Chem 49, 720, 1984) and then deprotected, as exemplified above.

(E) Synthesis of Compound C-2(d)



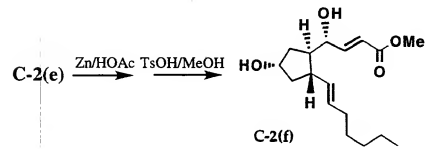
Di-THP-2(a) may be converted into the desired sulfonate using conventional procedures such as condensation of the alcohol with the appropriate sulfonyl chloride (see e.g. J Am Chem Soc 92, 553, 1970) and then deprotected, as exemplified above.

(F) Synthesis of Compound C-2(e)



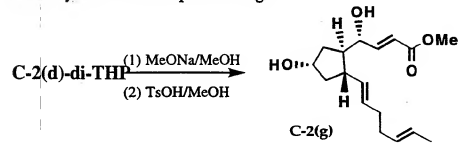
Di-THP-(C-2(d)) may be converted into the iodide, C-2(e), using conventional procedures such as treatment with sodium iodide/acetone (see e.g. J Chem Soc 92, 3650, 1950) and then deprotected, as exemplified above.

(G) **Synthesis of Compound C-2(f)**



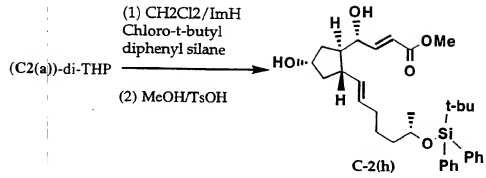
Iodo compounds such as C-2(e) may be converted into the corresponding alkyl compounds with zinc and acetic acid as illustrated above (see e.g. Org Syn Coll Vol 2, 320, 1943).

(H) **Synthesis of Compound C-2(g)**



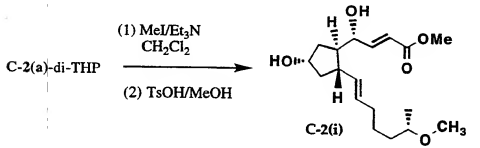
Protected tosylates such as C-2(d)-di-THP, as well as unprotected tosylates such as C-2(d), may be subjected to elimination conditions to form the alkenes, and then (in the former case) de-protected to yield compounds such as C-2(g). See e.g., J Chem Soc C 1115, 1967.

(I) **Synthesis of Compound C-2(h)**



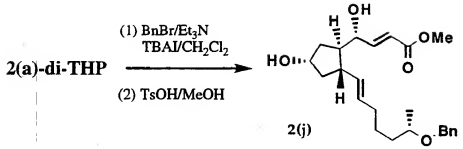
Di-THP-**C-2(a)** may be converted into the desired silyl derivative using conventional procedures such as condensation of the alcohol with the appropriate silyl chloride (see e.g. Can J Chem **53**, 2975, 1975) and then deprotected, as exemplified above.

(J) **Synthesis of Compound C-2(i)**



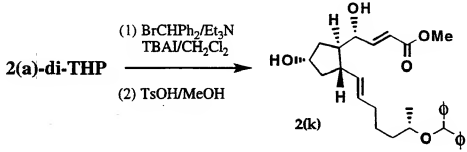
Di-THP-2(a) may be converted into the methyl ether with methyl iodide (see e.g. Tet Lett **30**, 641, 1989) and then deprotected, as exemplified above.

(K) Synthesis of Compound 2(j)



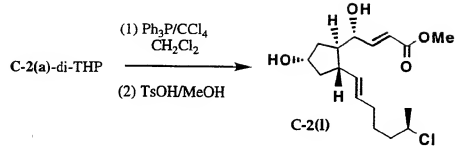
Di-THP-C-2(a) may be converted various ethers using the appropriate bromide and conventional procedures, (as illustrated above with benzyl bromide)(see e.g. Bull Chem Soc Japan 60 1529, 1987) and then deprotected, as exemplified above.

(L) Synthesis of Compound 2(k)



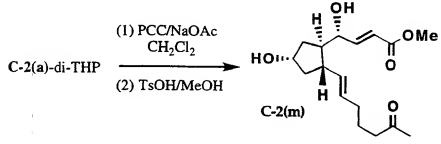
Di-THP-C-2(a) may be converted into the diphenylmethyl ether by the method of example 1(K) above, using diphenylmethylbromide in place of benzylbromide, followed by deprotection.

(M) Synthesis of Compound 2(l)



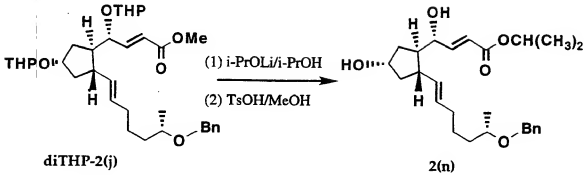
Chlorides can be prepared as illustrated above, using, e.g. the procedures of Can J Chem **46**, 86, 1968.

(N) Synthesis of Compound 2(m)



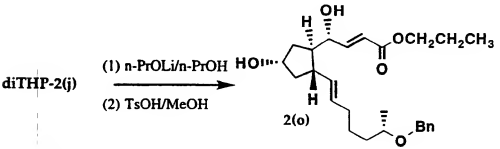
Di-THP-**C-2(a)** may be converted into the keto derivative using conventional procedures (see e.g. Tett Lett 3363, 1968) and then deprotected, as exemplified above.

(O) Synthesis of Compound 2(n)



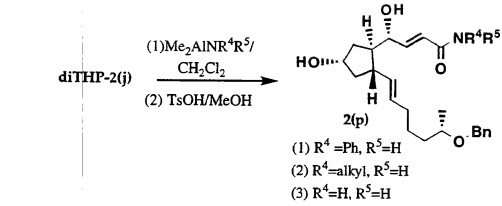
Various esters may be produced from compounds such as di-THP-2(j) by ester exchange using organolithium reagents containing the desired OR^1 group, followed by de-protection, as exemplified in the synthesis of Compound **C-2(n)** [and **C-2(o)** and **C-2(q)**, below]. See J Chem Soc, Chem Comm 695, 1986.

(P) Synthesis of Compound 2(o)



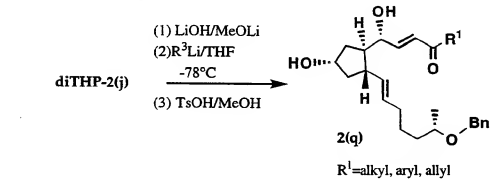
See Example 1(P) above.

(Q) Synthesis of Compound 2(p)



Various amides may be produced from esters such as di-THP-2(j) using conventional methods, including the use of aluminum reagents, as exemplified above (see e.g. Tett Lett 4171, 1977), followed by deprotection.

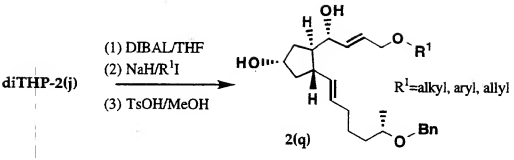
(R) Synthesis of Compound 2(q)



R^1=alkyl, aryl, allyl

Keto compounds may be prepared by conventional methods, including the route identified above. See e.g. Org React 18 1, 1970.

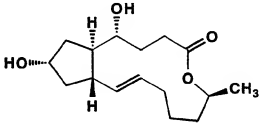
(S) Synthesis of Compound 2(r)



Allylic ethers, including those in which R^1 is methyl or benzyl, for example can be prepared from di-THP-2(j) by the route identified above. See e.g. J Am Chem Soc **107** 1777, 1985.

Example C-2: Synthesis of 2,3 dihydro- compounds

(a) Preparation of 2,3-dihydro-BFA **(III)**



III

The 2,3-dihydro derivative **(III)** can be synthesized using either of the two following methods.

Method 1

BFA (0.5 gram) is added to ethanol (16 mL) and stirred under a nitrogen atmosphere at room temperature until the BFA is dissolved. Thiophenol (0.29 gram) is added to this solution of dissolved BFA, followed by the addition of pyrrolidine (0.13 gram). The solution is stirred for 25 hours, at room

temperature. The reaction mixture is then diluted with methylene chloride and sequentially extracted with 0.05 N HCl, 0.05 N NaOH and brine. The organic layer is then dried over sodium sulfate, filtered and concentrated to an oil.

The above oil is taken into ethanol (20 mL) containing 5 grams of Raney Nickel catalyst (50% in water). The resulting slurry is stirred 4 hours at room temperature, and heated to reflux for an additional 4 hours. The catalyst is removed by filtration and the filtrate is diluted with methylene chloride and extracted sequentially with 0.5 N HCl, 0.5 N NaOH, and brine as described above. The organic layer is dried over sodium sulfate, filtered and concentrated to give the product as a solid.

Method 2

BFA (0.135 gram) is dissolved in methanol (4 mL) and stirred under a nitrogen atmosphere at room temperature until the BFA is dissolved. To this solution of dissolved BFA is added magnesium turnings (0.069 gram). The mixture is again stirred at room temperature until all of the magnesium metal is consumed (16 hours). The resultant turbid solution is poured into 1N hydrochloric acid (50 mL) and the solution is extracted three times with of ethyl acetate. The organic layers are combined and washed successively with water (50 mL) and brine (50 mL), dried over sodium sulfate, filtered and concentrated to a heavy clear oil.

The structure of **(I)**, made by either method 1 or method 2, was confirmed by NMR spectroscopy.

(b) Synthesis of 2,3-dihydro compounds

2,3-dihydro BFA may be used in place of BFA in the syntheses decribed herein.

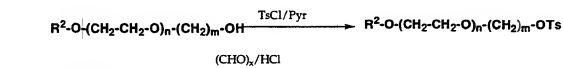
Example C-3: Use of alternative protecting groups

Other protecting groups for the hydroxyl groups at carbons 6 and 7 (per BFA numbering), e.g. MEM or dimethyl-t-bu-silyl groups, may be used in the synthetic schemes described herein so long as they are durable to the subsequent transformation conditions and may be removed without damaging other portions of the molecule.

Example D: Synthesis of Polyethers

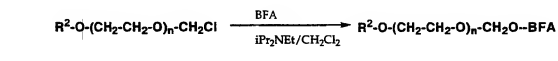
Example D-1. $R^2O\text{-}(CH_2CH_2O)_n\text{-}(CH_2)_m\text{-}E$

Various compounds of the formula $R^2O\text{-}(CH_2CH_2O)_n\text{-}(CH_2)_m\text{-}E$ where **E** is a halide (e.g. Cl or Br) or other convenient leaving group (such as tosylate) are commercially available or are otherwise known compounds and/or readily prepared, e.g. by the following schemes:



Example D-2. Attachment of $R^2O\text{-}(CH_2CH_2O)_n\text{-}(CH_2)_m\text{-}E$ to C7 position of BFA

$R^2O\text{-}(CH_2CH_2O)_n\text{-}(CH_2)_m\text{-}E$ compounds may be attached to the C7 position of BFA by conventional methods (See e.g. Corey and Wollenberg, Tet Lett, No. 51, 4701-4704 (1976)) as depicted below:



Example D-3. Further syntheses

BFA analogs which retain the 7-hydroxyl group may be used (with conventional protection and de-protection as appropriate) in place of BFA to produce analogous compounds containing the $R^2O\text{-}(CH_2CH_2)_n\text{-}(CH_2)_m\text{-}O\text{-}$ group at position 7. The resulting compounds may also be used in accordance with this invention.

E. Esters

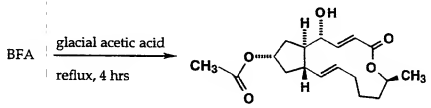
Example E-1. R^1-(C=O)-Q and R^1-(C=O)-O-(C=O)-R^1

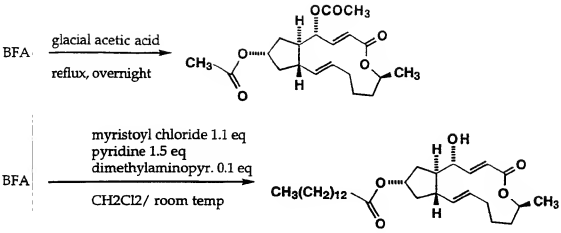
Various compounds of the formula R-(C=O)-Q where Q is a halide (e.g. Cl or Br) or hydroxy are commercially available or are otherwise known compounds and/or readily prepared. The same is true for the corresponding anyhydrides.

Example E-2. **Attachment of R^1-(C=O)-Q to position 7 of BFA with or without attachment to position 4.**

(1) Acid chlorides and anhydrides may be attached directly to the C7 position of BFA by classical procedures. For example, reaction with 1.1 eq of the anyhydride in CH_2Cl_2 with 1.5 equivalents of pyridine are usually complete within 3-4 hours at room temperature, although they can often be run overnight. Acid chlorides may be used in place of the anhydrides with the addition of catalytic amount of dimethylaminopyridine, for example. Alternatively, free acids [R^1-(C=O)-OH] may be attached to the 7-position using conventional reagents such as DCC (e.g. in methylene chloride with pyridine and DMAP, beginning at 0 °C and warming to room temperature over several hours) or the water soluble DDC or the Mukiyama reagent (a 2-halomethylpyridinium halide). The 4,7-disubstituted compounds may be prepared using at least a two-fold excess of the acid chloride, anhydride or acid.

(2) Exemplary syntheses





B. **Attachment of R^1-(C=O)-Qto C4 position of BFA**

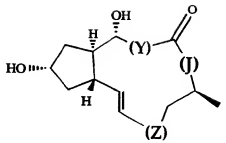
BFA may be protected at position 7 using conventional protecting groups such as MEM and the like and then reacted with the R^1-(C=O)-Q reagent under conventional conditions permitting attachment to the 4-hydroxy position. Deprotection at position 7 yields the mono-substituted compounds, modified at position 4.

Example E-3 Further syntheses

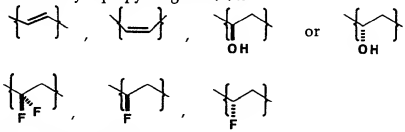
Compounds structurally related to BFA which retain the 4- and/or 7-hydroxyl groups may be used (with conventional protection and de-protection as appropriate) in place of BFA to produce analogous compounds containing the R^1-(C=O)-O- group at positions 4 and/or 7. The resulting compounds may also be used in accordance with this invention.

We claim:

1. A compound of the formula:



wherein **(J)** is O or substituted or unsubstituted N, **(Y)** is a carbon-carbon (trans) double bond or a cyclopropyl ring and **(Z)** is



or esters or polyethers thereof.

2. A method of inhibiting the transport of proteins from the endoplasmic reticulum in cells comprising administering to the individual an effective amount of a compound of claim 1.

3. A method of treating or preventing viral infection, fungal infection, tumor growth, untoward immune reactivity or pathological effects of a toxin, in an individual, which method comprises administering to the individual a compound of claim 1 in an amount effective therefor.

4. A pharmaceutical composition for treating or preventing viral infection, fungal infection, tumor growth, untoward immune reactivity or pathological effects of a toxin, which pharmaceutical composition comprises a compound of claim 1 and a pharmaceutically acceptable carrier.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/01656

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 31/365, 31/395; C07D 225/04, 313/00, 493/00
US CL :514/183, 450, 461; 540/461; 549/268, 270
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/183, 450, 461; 549/268, 270;

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DIE PHARMAZIE, Volume 47, No. 8, issued August 1992, B. Proska et al., "Oxidation of Brefeldin A", pages 582-584, entire document.	1-4
Y	US, A, 3,535,343 (CROSS ET AL.) 20 October 1970, entire document.	1-4

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be part of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 MAY 1995	15 JUN 1995

Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	BA K. TRINH
Facsimile No. (703) 305-3230	Telephone No. (703) 308-1235

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/01656

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/01656

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

I. Claims 1 and 2, drawn to compounds wherein (J) is oxygen and a method of use, class 549, subclass 268.

II. Claims 3 and 4, drawn to another method of use of compounds wherein (J) is oxygen, class 549, subclass 268.

III. Claim 1, drawn to compounds wherein (J) is nitrogen, class 540, subclass 461.

IV. Claims 1, 3 and 4 drawn to another method of use of compounds wherein (J) is nitrogen, class 540, subclass 461.

V. Claim 2, drawn to another method of use of compound where J is nitrogen.